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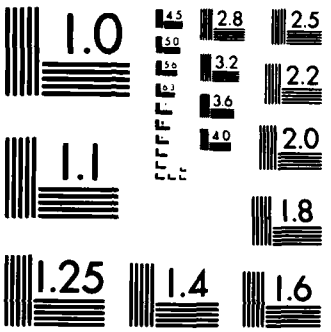

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Immunoenhancing drugs are under study both for their general effects on host immunity and their effects on immune interactions with microbial infections. These studies focused preferentially on defined synthetic single molecules, especially synthesized natural biological molecules or their active fragments.

We studied the mechanism of muramyl dipeptide in inducing hyporesponsiveness of spleen cells and hyperresponsiveness of lymph node cells to mitogens defining

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the cell populations and mediators involved in these effects.

In another study, murine macrophages from different anatomic compartments were compared for their fungicidal activity in different in vitro systems. Subsequently, the effect of a lipoidal amine immunomodulator on alveolar macrophage fungicidal activity was examined.

The mechanism of immunological activation of polymorphonuclear neutrophils for fungicidal activity was studied with respect to the oxidative mechanisms involved.

Recombinant gamma interferon was shown to activate macrophages for fungal killing.

Our work on immunomodulation and its mechanisms continues. The principal efforts of the past year, work in progress and future efforts are described below.

I

Earlier in the year we concluded the second phase of our studies on the mechanism of immunomodulation by muramyl dipeptide (MDP). We previously reported that a MDP treatment regimen (200 μg MDP per mouse, days -4, -3, -2, -1) that affords protection given prophylactically against several infectious agents also induces lymph node hyperplasia, and lymph node cell (LNC) hyperresponsiveness to mitogens, whereas spleen cells make depressed responses to mitogens. The purpose of the present work was to extend those studies and delineate cellular mechanisms involved in these phenomena. It was found that hyperresponsiveness of LNC was prolonged (7 days) post treatment; in contrast hyporesponsiveness of spleen cells was transient and rebounded by day 4 post treatment. Hyperresponsiveness of LNC and hyporesponsiveness of spleen cells actively enhanced or depressed normal lymphoid cell responses, respectively, in cell mixing experiments. Hyporesponsiveness of spleen cells was associated with the plastic nonadherent, non-B-cell fraction and nylon wool nonadherent subpopulations. Indomethacin (10^{-6}M) did not abrogate hyporesponsiveness of spleen cells. These data suggest that splenic suppressor T-cells result from MDP treatment and were responsible for spleen cell hyporesponsiveness. On the other hand, hyperresponsiveness of LNC was associated with the nylon wool adherent cell subpopulations and a higher percentage of nonspecific esterase positive cells. Hyporesponsiveness of spleen cells was associated with deficient production of interleukin-2 (IL-2), but not of interleukin-1 (IL-1). In contrast, hyperresponsiveness of LNC was not explained by enhanced IL-1 or IL-2 production.

II

Because of a need to extend our studies of effector cell function to alveolar macrophages, we performed further studies of alveolar macrophages in our killing systems with fungal targets. These studies extend our observations previously reported with these cells and Candida albicans (Amer. Rev. Resp. Dis. 127:110-112, 1983)

Murine bronchoalveolar macrophage (BAM) fungicidal activity against the yeast form of a virulent strain of Blastomyces dermatitidis was studied in the small volume wells of a Terasaki tray. In a four hour co-culture assay, significant killing (16-33%) of the fungus was demonstrated with resident BAM and with BAM obtained from mice rendered immune to lethal infection with B. dermatitidis. No significant differences between the activity of BAM from these two sources could be identified. Addition of 10% autologous normal or immune serum did not augment macrophage fungicidal activity. Simultaneous experiments with peritoneal macrophages (PM) also gave reproducible killing of the yeast form in wells of the Terasaki tray. However, PM studied in a standard microtiter plate assay showed no statistically significant fungicidal activity. On the other hand, BAM had similar fungicidal activity against B. dermatitidis in Terasaki and microtest plate wells. Modest fungicidal ability of resident BAM in immune mice against B. dermatitidis suggests that the striking resistance of immune mice to respiratory challenge is likely based on some augmentation of this first line of defense.

III

Our studies of the lipoidal amine synthetic immunomodulator CP46665 also continued. Our results with polymorphonuclear neutrophils (PMN) were reported previously. Further studies with BAM were performed, which failed to demonstrate a significant effect, not confirming our earlier promising results. These experiments were done at the apparent optimal dose of 5 mg/kg, in a single dose, 1 day prior to experiment, using Terasaki plates. The control is the diluent for the intravenous injection, intralipid.

TABLE 1

PERCENT KILLING OF CANDIDA

Experiment #	Intralipid	CP46665
1	10	43
2	17	47
3	18	18
4	22	20
5*	14	5
6	12	23

*Utilized log phase C. albicans, others used plateau phase

If we omit experiment 5, which was the only one not identical in technique to others, the data can be analyzed as follows. The means ± S.D. are 16 ± 5% for IL and 30 ± 14% for CP, which (apparently because of the large CP S.D.) is $p > 0.05$.

We also performed experiments when the dose was given in 2 consecutive days prior to the experiment. These 1 dose and 2 dose regimens, with sampling the effect on cells the following day, appeared optimal in early dose-finding experiments.

TABLE 2

PERCENT KILLING OF CANDIDA

Experiment #	IL	CP
7	5	11
8	3	15
9	6	3
10	10	15

Applying the same analysis, the means ± S.D. are 6 ± 3% and 11 ± 6%. Thus, even though 3 of 4 experiments show and edge for CP, the p is still >0.05.

IV

Recent studies have focused on the mechanism of the interaction between the T-cell mediated immune system and polymorphonuclear leukocytes (PMN) we previously described. We have shown, as previously described, using fungi (three different genera) as the target organisms, that the PMN can be modulated by lymphokines to new or enhanced levels of killing ability.

PMN induced locally in immune mice by intraperitoneal injection of antigen exhibit enhanced fungicidal activity compared to PMN elicited with thioglycollate. The mechanism of the differences in these PMN populations was studied. Sublethal infection was used to produce immunity to B. dermatitidis. A correlation was sought between ability of PMN to kill (or not kill) B. dermatitidis and production of the oxidative burst, as measured by luminol-enhanced chemiluminescence. Although elicited PMN cocultured with C. albicans produced a burst of chemiluminescence and were candidacidal, killing did not occur when the PMN were co-cultured with B. dermatitidis. Lack of killing of B. dermatitidis by elicited PMN correlated with lack of stimulation of a brisk oxidative burst. In contrast to elicited PMN, PMN induced by B. dermatitidis antigen responded to this fungus with a burst of chemiluminescence and a significant reduction of inoculum colony forming units (80%). Furthermore, these PMN when co-cultured with C. albicans produced an enhanced burst of chemiluminescence, and killing was enhanced compared to elicited PMN, e.g. 86 vs. 58%. The chemiluminescence burst and killing of B. dermatitidis by antigen-induced PMN was abrogated in the presence of catalase, implying a critical role for hydrogen peroxide. Partial but significant depression of chemiluminescence and killing in the presence of dimethyl sulfoxide, a hydroxyl radical scavenger, identified hydroxyl radical, or its metabolites, as toxic product(s) responsible for a significant fraction of fungicidal activity. These results indicate that the metabolic activity and microbicidal activity of PMN can be altered (enhanced) at the site of an immunological reaction, and thus could constitute an important factor in resistance.

V

Although our earlier reported studies indicated no beneficial effect of recombinant alpha-interferon on macrophages with respect to fungal targets, and in some instances a deleterious effect (these studies included human IF, C. albicans, human PMN and monocytes, and phagocytosis and killing), further studies with gamma interferon were indicated.

Recombinant murine gamma-interferon (IF) and supernatants from concanavalin A (ConA) stimulated mouse spleen cells (ConA-Sup) were tested for their ability to activate resident murine PM (peritoneal macrophages) (90% esterase positive) for fungicidal activity. PM monolayers pulsed overnight with IF had significantly enhanced fungicidal activity against C. albicans. (44 ± 12% vs. 0.0%, mean ± SD of 3 experiments) and B. dermatitidis (34 ± 1% vs. 3 ± 3%). The effect was dose dependent; however, less IF (10 U/ml) was required to optimally activate PM to kill phagocytizable C. albicans than for nonphagocytizable B. dermatitidis (10,000 U/ml). ConA alone (5-10 mcg/ml) enabled PM to kill C. albicans but not B. dermatitidis. ConA-Sup activated PM for fungicidal activity against B. dermatitidis. ConA-Sup collected at 24 hrs. best activated PM for killing (30 ± 3%) compared to those at 48 or 72 hrs. Capacity to activate PM for killing C. albicans or B. dermatiti was abrogated when IF was incubated with anti-mouse IF monoclonal antibody. C. albicans killing was still induced after neutralizing ConA in ConA-Sup by absorption with Sephadex G-10 or incubation with alpha-methyl mannoside. Lipopolysaccharide (0.001-10 mcg/ml) failed to consistently activate PM for fungal killing. These data show that IF can have an immunoregulatory role in macrophage defense against these fungal pathogens, and may have therapeutic potential.

Future studies planned. With respect to section I above, these may include (a) further definition of the cell subsets affected by MDP, and (b) determining the mechanism of MDP protection against Blastomyces infection in vivo, by assaying humoral immunity and cell-mediated immunity (including macrophage) function in our model after in vivo MDP administration. With respect to section III, we will be completing some in vivo studies with CP 46665. With respect to section IV, the studies will progress in the following way. One avenue of research will pursue the biochemical mechanisms in the PMN consequent to immune activation of PMN's via lymphokines that result in

increased or new killing power. The second will be to characterize the lymphokine involved. The third avenue will attempt to define biological modifiers, produced by recombinant DNA or hybridoma techniques, which may produce the same effect. Prime candidates are gamma interferon and interleukin-2. If this is uncovered, it could lead to a therapeutic approach to defense against fungal pathogens. The last avenue will be to explore the effect of other eliciting agents, which may cause release in vivo of inflammatory mediators that could result in enhanced killing compared to the results with thioglycollate, and metabolic effects and killing power comparable to elicitation in immune animals with antigen, as we have described. With respect to section V, we are interested in the biochemical mechanisms in the macrophage that correlate with the enhanced fungicidal activity following gamma interferon treatment.

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Dr. Jeannine A. Majde, Code 441CB
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Minneapolis, MN 55455

Dr. John D. Clements
Department of Microbiology
and Immunology
Tulane University Medical Ctr.
1430 Tulane Avenue
New Orleans, LA 70112

Francis A. Ennis, M.D.
Department of Medicine
University of Massachusetts
Medical School
55 Lake Avenue
Worcester, MA 01605

Dr. Edward A. Havell
Trudeau Institute
P.O. Box 59
Saranac Lake, NY 12983

Fred D. Finkelman, M.D.
Department of Medicine
Uniformed Services University
of the Health Sciences
4301 Jones Bridge Road
Bethesda, MD 20814

Dr. Arthur G. Johnson
Department of Medical
Microbiology and Immunology
University of Minnesota
School of Medicine
2205 East 5th Street
Duluth, MN 55812

Dr. Matthew J. Kluger
Department of Physiology
University of Michigan Med. School
7620 Medical Science II Bldg.
Ann Arbor, MI 48109

Dr. Philip Lake
Immunologic Oncology Division
Lombardy Cancer Center
Georgetown Univ. School of Med
Washington, DC 20007

Dr. Vijaya Manohar
Borriston Laboratories, Inc.
5050 Beech Place
Temple Hills, MD 20748

W. John Martin, M.D., Ph.D.
Laboratory, Dept. of Medicine
Naval Hospital
National Naval Medical Center
Bethesda, MD 20814

Dr. Ernest D. Marquez
Bioassay Systems Corporation
225 Wildwood Avenue
Woburn, MA 01801

Dr. Robert I. Mishell
Dept. of Microbiology &
Immunology
Univ. of California, Berkeley
Berkeley, CA 94720

James J. Mond, M.D.
Department of Medicine
Uniformed Services University
of the Health Sciences
4301 Jones Bridge Road
Bethesda, MD 20814

Dr. Page S. Morahan
Department of Microbiology
Medical College of Pennsylvan
3300 Henry Avenue
Philadelphia, PA 19129

Dr. Donna S. Sieckmann
Infectious Diseases Program Center
Naval Medical Research Inst.
National Naval Medi enter
Bethesda, MD 20814

Dr. Alan L. Schmaljohn
Department of Microbiology
University of Maryland
School of Medicine
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David A. Stevens, M.D.
Department of Medicine
Santa Clara Valley Medical Center
Stanford University
751 S. Bascom Avenue
San Jose, CA 95128

Dr. Barnet M. Sultzer
Department of Microbiology &
Immunology
Downstate Medical Center
450 Clarkson Avenue
Brooklyn, NY 11203

Dr. Alvin L. Winters
Department of Microbiology
University of Alabama
University, AL 35486

Dr. Phyllis R. Strauss
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Northeastern University
360 Huntington Avenue
Boston, MA 02115

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